Chapter 17
Glycolysis

SUMMARY

Section 17.1

- In glycolysis, glucose is converted to pyruvate in a multistep pathway.
- When pyruvate is formed, it can be converted to carbon dioxide and water in aerobic reactions. It can also be converted to lactate under anaerobic conditions or, in some organisms, to ethyl alcohol.
- Glucose is converted to pyruvate in a series of 10 reactions, only one of which is an oxidation.

Section 17.2

- In the first stages of glycolysis, glucose is converted to two molecules of glyceraldehyde- 3-phosphate.
- The key intermediate in this series of reactions is fructose-1,6-bisphosphate. The reaction that produces this intermediate is a key control point of the pathway, and the enzyme that catalyzes it, phosphofructokinase, is subject to allosteric regulation.

Section 17.3

- In the final stages of glycolysis, two molecules of pyruvate are produced for each molecule of glucose that entered the pathway.
- These reactions involve electron transfer (oxidation–reduction) and the net production of two ATP for each glucose.

Section 17.4

- Pyruvate is converted to lactate in anaerobic tissues, such as actively metabolizing muscle. NAD+ is recycled in the process.
- In some organisms, pyruvate is converted to ethanol in a process requiring thiamine pyrophosphate as a coenzyme.
- Cancer cells rely heavily on aerobic glycolysis, referred to as the Warburg effect

Section 17.5

- Glycolysis is an exergonic process, releasing 73.4 kilojoules for every mole of glucose converted to two moles of pyruvate, accompanied by phosphorylation of two moles of ADP to ATP.
- Without the production of ATP, glycolysis would be even more strongly exergonic.

Section 17.6

- There are three major control points of glycolysis
- The first is the conversion of glucose to glucose 6-phosphate
- The second and most important is the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate.
- The third is the conversion of PEP to pyruvate
- In addition to the enzymatic control, there is also control via hormones.

LECTURE NOTES

Glycolysis is, arguably, is best understood biochemical pathway, and thus serves an excellent introduction to metabolism in general. A general description of the enzymes and reactions involved will probably take up two lectures, dependent upon the depth of coverage of specific mechanisms and energetics. While introduced here, detailed discussion of regulatory mechanisms is left for chapter 18.

LECTURE OUTLINE

I. Overview of glycolysis
    A. 1 glucose converted to 2 pyruvate
    B. Net gain of 2 ATP
    C. Anaerobic pathway
II. Reaction summary
    A. Phosphorylation
    B. Isomerization
    C. Phosphorylation
    D. Cleavage
    E. Isomerization
    F. Oxidation
    G. Phosphate transfer
    H. Isomerization
    I. Dehydration
    J. Phosphate transfer
III. Preparation phase
    A. Phosphorylation
        1. Coupling of exergonic ATP hydrolysis to endergonic phosphorylation of glucose
        2. Hexokinase
        3. Glucose-6-phosphate inhibition
        4. Glucokinase action
        5. Conformational changes in hexokinase
    B. Isomerization
        1. Glucose-6-phosphate isomerase
        2. Conversion from aldose to ketose
    C. Phosphorylation
        1. Similarity to step 1
        2. Committed step
        3. Phosphofructokinase
        4. Allosteric regulation

5. Isozymes
6. Inhibition by ATP

D. Cleavage
1. Generation of two 3-carbon fragments
2. Aldolase

E. Isomerization
1. Triosephosphate isomerase
2. Comparison of ΔG with overall ΔG of pathway

IV. Payoff phase

A. Oxidation
1. Phosphate addition
2. Electron transfer — aldehyde to carboxylic acid, NAD+ to NADH
3. No ATP involvement
4. Combination of $\Delta G^{\circ\prime}$ of oxidation and $\Delta G^{\circ\prime}$ of phosphorylation
5. Glyceraldehyde-3-phosphate dehydrogenase mechanism

B. Phosphate transfer
1. Production of ATP
2. Phosphoglycerate kinase
3. Substrate-level phosphorylation
4. Coupling of exergonic BPG hydrolysis to endergonic phosphorylation of ADP

C. Isomerization
1. Phosphoglyceromutase
2. Transfer of phosphate from C-3 to C-2

D. Dehydration
1. Enolase
2. Loss of water

E. Phosphate transfer
1. Substrate-level phosphorylation
2. Coupling of exergonic PEP hydrolysis to endergonic phosphorylation of ADP
3. Pyruvate kinase

V. Glycolytic control points

ANSWERS TO PROBLEMS

17.1 The Overall Pathway of Glycolysis

1. Reactions that require ATP: phosphorylation of glucose to give glucose-6-phosphate and phosphorylation of fructose-6-phosphate to give fructose-1,6-bisphosphate. Reactions that produce ATP: transfer of phosphate group from 1,3-bisphosphoglycerate to ADP and transfer of phosphate group fromphosphoenolpyruvate to ADP. Enzymes that catalyze reactions requiring ATP: hexokinase, glucokinase, and phosphofructokinase. Enzymes that catalyze reactions producing ATP: phosphoglycerate kinase and pyruvate kinase.
2. Reactions that require NADH: reduction of pyruvate to lactate and reduction of acetaldehyde to ethanol. Reactions that require NAD^+: oxidation of

glyceraldehyde-3-phosphate to give 1,3- diphosphoglycerate. Enzymes that catalyze reactions requiring NADH: lactate dehydrogenase and alcohol dehydrogenase. Enzymes that catalyze reactions requiring NAD^+: glyceraldehyde-3-phosphate dehydrogenase.

3. Pyruvate can be converted to lactate, ethanol, or acetyl-CoA.

17.2 Conversion of six-carbon glucose to three-carbon glyceraldehydes-3-phosphate

4. Aldolase catalyzes the reverse aldol condensation of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.
5. Isozymes are oligomeric enzymes that have slightly different amino acid compositions in different organs. Lactate dehydrogenase is an example, as is phosphofructokinase.
6. Isozymes allow for subtle control of the enzyme to respond to different cellular needs. For example, in the liver, lactate dehydrogenase is most often used to convert lactate to pyruvate, but the reaction is often reversed in the muscle. Having a different isozyme in the muscle and liver allows for those reactions to be optimized.
7. Fructose-1,6-bisphosphate can only undergo the reactions of glycolysis. The components of the pathway up to this point can have other metabolic fates.
8. Add the $\Delta G^{\circ'}$ mol^{-1} values for the reactions from glucose to glyceraldehyde-3-phosphate. The result is 2.5 kJ mol^{-1} = 0.6 kcal mol^{-1}
9. The two enzymes can have different tissue locations and kinetic parameters. The glucokinase has a higher K_M for glucose than hexokinase. Thus, under conditions of low glucose, the liver does not convert glucose to glucose-6-phosphate, using the substrate that is needed elsewhere. When the glucose concentration is much higher, however, glucokinase helps phosphorylate glucose so that it can be stored as glycogen.
10. Individuals who lack the gene that directs the synthesis of the M form of the enzyme can carry on glycolysis in their livers but experience muscle weakness because they lack the enzyme in muscle.
11. The hexokinase molecule changes shape drastically on binding to substrate, consistent with the induced-fit theory of an enzyme adapting itself to its substrate.
12. ATP inhibits phosphofructokinase, consistent with the fact that ATP is produced by later reactions of glycolysis.

17.3 Glyceraldehyde-3-phosphate is converted to pyruvate

13. From the point at which aldolase splits fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate; all reactions of the pathway are doubled (only the path from one glyceraldehyde-3-phosphate is usually shown).
14. NADH-linked dehydrogenases: Glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase, and alcohol dehydrogenase.
15. The free energy of hydrolysis of a substrate is the energetic driving force in substrate-level phosphorylation. An example is the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate.
16. The control points in glycolysis are the reactions catalyzed by hexokinase,

phosphofructokinase, and pyruvate kinase.

17. Hexokinase is inhibited by glucose-6-phosphate. Phosphofructokinase is inhibited by ATP and citrate. Pyruvate kinase is inhibited by ATP, acetyl-CoA, and alanine. Phosphofructokinase is stimulated by AMP and fructose-2,6-bisphosphate. Pyruvate kinase is stimulated by AMP and fructose-1,6-bisphosphate.
18. The part of the active site that binds to NADH would be the part that is most conserved, since many dehydrogenases use that coenzyme.
19.

(a) Using a high-energy phosphate to phosphorylate a substrate.
(b) Changing the form of a molecule without changing its empirical formula (i.e., replacing one isomer with another).
(c) Performing an aldol cleavage of a sugar to yield two smaller sugars or sugar derivatives.
(d) Changing the oxidation state of a substrate by removing hydrogens while simultaneously changing the oxidation state of a coenzyme (NADH, $FADH_2$, etc.).

20. An isomerase is a general term for an enzyme that changes the form of a substrate without changing its empirical formula. A mutase is an enzyme that moves a functional group, such as a phosphate, to a new location in a substrate molecule.
21. The reaction of 2-phosphoglycerate to phosphoenolpyruvate is a dehydration (loss of water) rather than a redox reaction.
22. Carbon-1 of glyceraldehyde is the aldehyde group. It changes oxidation state to a carboxylic acid, which is phosphorylated simultaneously.
23. ATP is an inhibitor of several steps of glycolysis as well as other catabolic pathways. The purpose of catabolic pathways is to produce energy, and high levels of ATP mean the cell already has sufficient energy. Glucose-6-phosphate inhibits hexokinase and is an example of product inhibition. If the glucose-6-phosphate level is high, it may indicate that sufficient glucose is available from glycogen breakdown or that the subsequent enzymatic steps of glycolysis are going slowly. Either way, there is no reason to produce more glucose-6-phosphate. Phosphofructokinase is inhibited by a special effector molecule, fructose-2,6-bisphosphate, whose levels are controlled by hormones. It is also inhibited by citrate, which indicates that there is sufficient energy from the citric acid cycle, probably from fat and amino acid degradation. Pyruvate kinase is also inhibited by acetyl-CoA, the presence of which indicates that fatty acids are being used to generate energy for the citric acid cycle. The main function of glycolysis is to feed carbon units to the citric acid cycle. When these carbon skeletons can come from other sources, glycolysis is inhibited to spare glucose for other purposes.
24. There would be 15 possible isozymes of LDH, combining three different subunits into combinations of four. Besides the five isozymes containing only M and H, there would also be C_4, CH_3, C_2H_2, C_3H, CH_2M, C_2HM, C_3M, CHM_2, C_2M_2, and CM_3.
25. Glutamic acid has an acidic side chain with a pKa of 4.25. Therefore, it would be

negatively charged at pH 8.6, and the H subunit would move more toward the anode (+) than the M subunit. Thus, LDH 1, which is H_4, would move the farthest. LDH 5, which is M_4, would move the least, with the other isozymes migrating between those two extremes proportional to their H content.

26. With few exceptions, a biochemical reaction typically results in only one chemical modification of the substrate. Accordingly, several to many steps are needed to reach the ultimate goal.

27. The enzyme contains a phosphate group on a suitable amino acid, such as serine, threonine, and histidine. The substrate donates its phosphate group from the C-3 position to another amino acid on the enzyme, subsequently receiving the one that started out on the enzyme. Thus, the ^{32}P that was on the substrate is transferred to the enzyme, while an unlabeled phosphorus is put on the C-2 position.

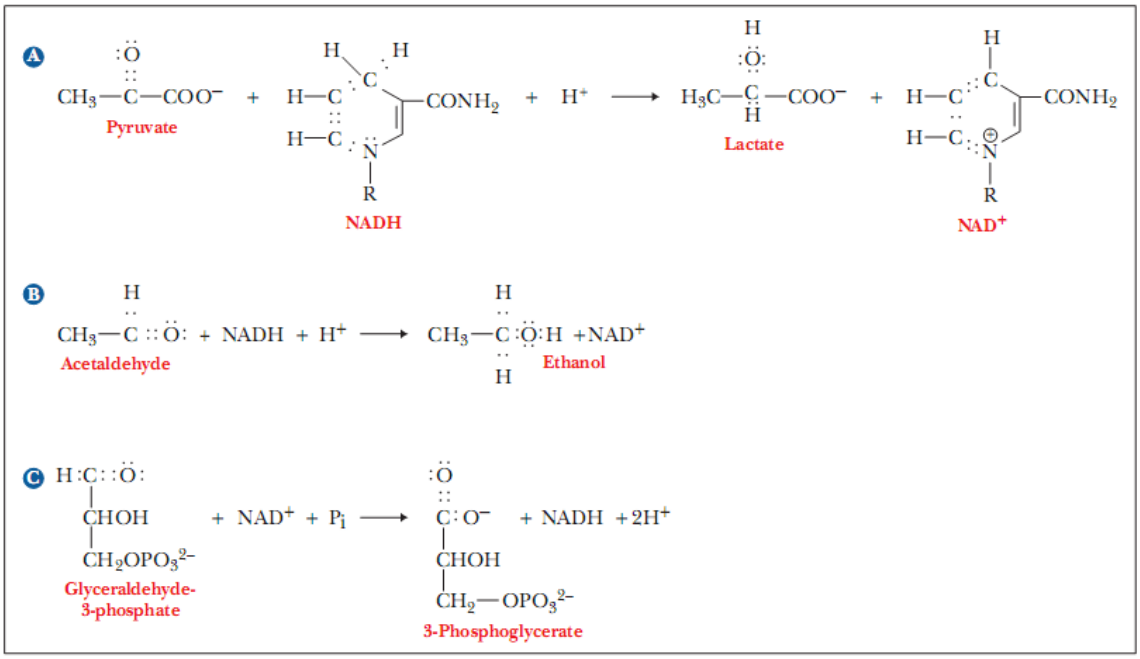
17.4 Structures and Functions of Polysaccharides

28. The bubbles in beer are CO_2, produced by alcoholic fermentation. Tired and aching muscles are caused in part by a buildup of lactic acid, a product of anaerobic glycolysis.

29. The problem with lactic acid is that it is an acid. The H^+ produced from lactic acid formation causes the burning muscle sensation. Sodium lactate is the conjugate weak base of lactic acid. It is reconverted to glucose by gluconeogenesis in the liver. Giving sodium lactate intravenously is a good way to supply an indirect source of blood glucose.

30. The purpose of the step that produces lactic acid is to reduce pyruvate so that NADH can be oxidized to NAD^+, which is needed for the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase.

31.



32. Thiamine pyrophosphate is a coenzyme in the transfer of two-carbon units. It is required for catalysis by pyruvate decarboxylase in alcoholic fermentation.
33. The important part of TPP is the five-membered ring, in which a carbon is found between a nitrogen and a sulfur. This carbon forms a carbanion and is extremely reactive, making it able to perform a nucleophilic attack on carbonyl groups, leading to decarboxylation of several compounds in different pathways.
34. Thiamine pyrophosphate is a coenzyme required in the reaction catalyzed by pyruvate carboxylase. Because this reaction is a part of the metabolism of ethanol, less will be available to serve as a coenzyme in the reactions of other enzymes that require it.
35. Animals that have been run to death have accumulated large amounts of lactic acid in their muscle tissue, accounting for the sour taste of the meat.
36. Conversion of glucose to lactate rather than pyruvate recycles NADH.
37. This is possible, and it is done. These poisons also affect other tissues, including skin, hair, cells of the intestinal lining, and especially the immune system and red blood cells. People on chemotherapy are usually more susceptible to infectious diseases than healthy people and are often somewhat anemic.
38. The Warburg effect is the high level of glycolysis in cancer cells, giving rise to pyruvate, followed by lactic acid fermentation. This effect is observed even at high levels of oxygen, where further oxidation to carbon dioxide and water is expected. This is one of the many aspects of glycolysis, which is the main subject of this chapter.
39. Research is in progress to modify pyruvate kinase isozymes typical of cancer cells to resemble those of normal cells. The goal is to redirect metabolism to that of normal cells, rather than cancer cells.

17.5 Energy Production in Glycolysis

40. The energy released by all the reactions of glycolysis is 184.5 kJ mol glucose–1. The energy released by glycolysis drives the phosphorylation of two ADP to ATP for each molecule of glucose, trapping 61.0 kJ mol glucose–1. The estimate of 33% efficiency comes from the calculation (61.0/184.5) x 100 = 33%.
41. There is a net gain of two ATP molecules per glucose molecule consumed in glycolysis.
42. The gross yield is four ATP molecules per glucose molecule, but the reactions of glycolysis require two ATP per glucose.
43. The reactions catalyzed by hexokinase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerokinase, and pyruvate kinase.
44. The steps catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.
45.

$$\text{Phosphoenolpyruvate} \rightarrow \text{pyruvate} + P_i$$
$$\Delta G^{\circ\prime} = -61.9 \text{ kJ mol}^{-1} = -14.8 \text{ kcal mol}^{-1}$$
$$\text{ADP} + P_i \rightarrow \text{ATP}$$
$$\Delta G^{\circ\prime} = 30.5 \text{ kJ mol}^{-1} = 7.3 \text{ kcal mol}^{-1}$$
$$\text{Phosphoenolpyruvate} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP}$$
$$\Delta G^{\circ\prime} = -31.4 \text{ kJ mol}^{-1} = -7.5 \text{ kcal mol}^{-1}$$

46. The net yield of ATP from glycolysis is the same, two ATP, when either of the

three substrates is used. The energetics of the conversion of hexoses to pyruvate are the same, regardless of hexose type.

47. Starting with glucose-1-phosphate, the net yield is three ATP, because one of the priming reactions is no longer used. Thus, glycogen is a more efficient fuel for glycolysis than free glucose.

48.

Phosphoenolpyruvate + ADP → Pyruvate + ATP	$\Delta G^{\circ\prime} = -31.4$ kJ/mol
ADP + P_i → ATP	$\Delta G^{\circ\prime} = 30.5$ kJ/mol
Sum	$\Delta G^{\circ\prime} = -0.9$ kJ/mol

Thus, the reaction is thermodynamically possible under standard conditions.

49. No, the reaction shown in Question 48 does not occur in nature. We can assume that no enzyme evolved that could catalyze it. Nature is not 100% efficient.

50. A positive $\Delta G^{\circ\prime}$ does not necessarily mean that the reaction has a positive ΔG. Substrate concentrations can make a negative ΔG out of a positive $\Delta G^{\circ\prime}$.

51. The entire pathway can be looked at as a large coupled reaction. Thus, if the overall pathway has a negative ΔG, an individual step may be able to have a positive ΔG, and the pathway can still continue.

17.6 Control of Glycolysis

52. The formation of fructose-1,6-bisphosphate is the committed step in the glycolytic pathway. It is also one of the energy-requiring steps of the pathway.

53. Glucose-6-phosphate inhibits hexokinase, the enzyme responsible for its own formation. Because G-6-P is used up by additional reactions of glycolysis, the inhibition is relieved.

54. Hormones exercise another level of control over metabolism, beyond that of allosteric and feedback control. Glycolysis can certainly be affected in this manner. The effect of insulin on carbohydrate metabolism is well known.

55. Exerting control at the end of a pathway is a good example of feedback control, so it is reasonable to expect it.